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EFFECT OF CERTAIN SOLIDS UPON THE GROWTH OF SEEDLINGS IN WATER CULTURES.[1]

J. F. BREAZEALE.

(WITH FOUR FIGURES)

In certain investigations on the growth of wheat seedlings in aqueous extracts of soil, it was observed that the growth of these plants was greatly accelerated by the presence in the medium of undissolved calcium carbonate. That the observed acceleration was not due to an increase in dissolved calcium was apparent from the fact that the presence of other slightly soluble salts of this element failed to produce any response. It appeared possible that the effect of calcium carbonate might be due to its taking up some injurious substance present in the extract. This was suggested by NÄGELI'S well-known discovery[2] that water, which is toxic to algae because of minute traces of metals, can be improved by placing in it such insoluble bodies as graphite, paraffin shavings, or torn filter paper. It was determined to try other slightly soluble compounds which might remove from solution small amounts of solutes, either by chemical action or mechanically. The results of this investigation make up the present paper.

The Russian variety of wheat known as "Chul," obtained from Arizona, was used in most of these experiments. The seedlings were germinated in sand and then grown in water cultures in large-mouthed black bottles of about 60^{cc} capacity. They were fixed in cork stoppers, four in a bottle, in the manner described by WHITNEY and CAMERON[3] for cultures of this kind, so that the roots were submerged in the solution while the seeds were just above its surface. The solutions were always aerated by violent and repeated shaking before the cultures were started. During the growth of the plants the bottles were weighed in groups of three at intervals of three or four days, and the water lost was replaced with distilled water. The manner of fixing the seedlings practically prevents water loss, except through the transpiration of the plants. The work of LIVINGSTON[4] indicates that total loss by transpiration for a period of two or three weeks furnishes a fair criterion for comparison of the growth of different cultures of wheat grown in this manner. The transpiration figures are used in this way in the experiments here given. The work was carried on in a greenhouse with a temperature of 15 to 25° C.

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[1] Published by permission of the Secretary of Agriculture.

[2] Nägeli, C. von, Ueber oligodynamische Erscheinungen in lebenden Zellen. Denkschr. Schweiz. Naturforsch. Gesell. 33:1. 1893.

[3] WHITNEY, M., and CAMERON, F. K., Investigations in soil fertility. U. S. Dept. Agric., Bureau of Soils, Bull. 23. 1904.

For Experiments I to III a soil extract from poor Leonardtown loam, collected near Leonardtown, Md., was used. It was prepared by stirring the soil for three minutes with water in amount equal to twice its air dry weight, allowing it to stand twenty minutes, and then filtering through a clean Pasteur-Chamberland filter tube, in the manner described by WHITNEY and CAMERON.[5]

In Experiment I the solids used were calcium carbonate, tricalcium phosphate, ferric hydrate and aluminum hydrate. Ferric hydrate was prepared by precipitation from the chlorid with ammonia, followed by thorough washing with hot water. It was transferred moist to the culture media. Aluminum hydrate was prepared in a similar way from the sulfate. Data for this and the three following experiments are given in Table I. The percentage increase in transpiration for each solid is computed by considering the transpiration from the untreated extract as unity.

All of the solids accelerated growth, as is shown by the transpiration figures. But in the case of the ferric hydrate the root growth was accelerated to a much greater extent than that of the tops. The roots of the culture with this substance were much longer than those of the other cultures. It is evident here that root growth was accelerated without a corresponding increase in transpiration.

Experiment II comprised, besides calcium carbonate and ferric hydrate, carbon black (prepared from burning petroleum, and thoroughly washed), magnesium carbonate, and barium carbonate. The small amounts of water transpired are due to cloudy weather.

The plants of this series are shown in *fig. 1*, the numbers in the figure corresponding to the culture numbers given in parentheses in the table. It will be noticed that carbon black shows the same tendency to produce abnormal root growth as does ferric hydrate, but to a less marked degree.

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[4] LIVINGSTON, B. E., Relation of transpiration to growth in wheat. BOT. GAZETTE 40:178–195. 1905.

[5] WHITNEY, M., and CAMERON, F. K., The chemistry of the soil as related to crop production. U. S. Dept. Agric., Bureau of Soils, Bull. 22:16 ff. 1903.

Experiment III included very finely pulverized quartz flour, as well as ferric hydrate and carbon black. The two last named bodies showed here the same abnormal acceleration of root growth as was previously observed, but quartz flour, although it improved the general growth of the plants, produced no such effect.

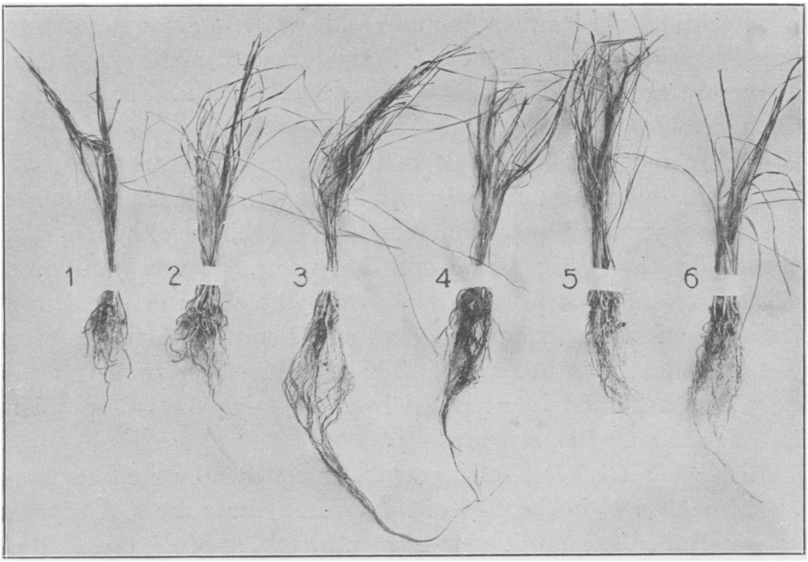

FIG. 1.—Experiment II; 24 wheat plants grown 19 days. 1, Extract of Leonardtown loam; 2, the same with calcium carbonate; 3, the same with ferric hydrate; 4, the same with carbon black; 5, the same with magnesium carbonate; 6, the same with barium carbonate.

The experiment with ferric hydrate and carbon black has been repeated many times with extract of Leonardtown loam, as well as that of other soils, and always with the same result. In some cases acceleration of root growth is more marked with carbon black than with ferric hydrate, but usually the reverse is true.

Experiment IV was carried out with an aqueous extract, prepared as above, from Miami silt loam collected at the Rhode Island Experiment Station, at Kingston, R. I. This soil had been in hoed crops for ten years without fertilizer, and was acid to litmus paper. To make absolutely sure that the effect of carbon black was not due to any substance added with it, the distilled water for the soil extract was shaken with the solid carbon black and filtered through a Pasteur-Chamberland tube before being used. Ferric hydrate, carbon black, and a mixture of these two bodies, together with calcium carbonate, were used in this case. The plants are shown in *fig. 2*.

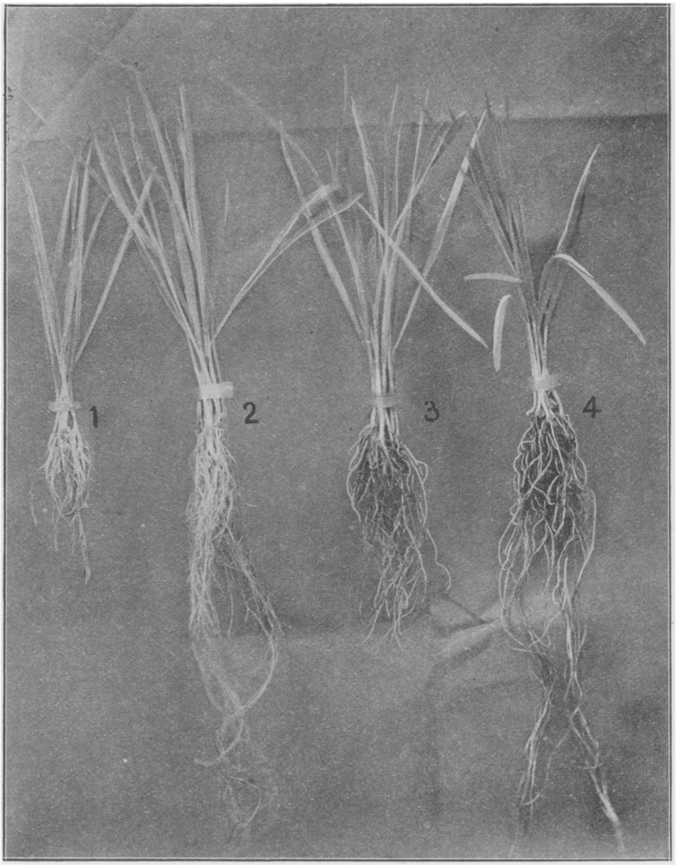

FIG. 2.—Experiment IV; 36 wheat plants grown 13 days. 1, extract of Miami silt loam; 2, the same with ferric hydrate; 3, the same with carbon black; 4, the same with ferric hydrate, carbon black, and calcium carbonate.

From this experiment and others of similar nature it seems clear that the effect of the carbon black is not due to any stimulating substance which it carries into the medium. Other experiments have shown that no acceleration of growth is obtained with the addition of iron salts to the extract of poor Leonardtown loam. Therefore, a slight increase in dissolved iron cannot be the cause of the acceleration noted in the case of this hydrate. It seemed probable that ferric hydrate and carbon black had their effect through an active removal, perhaps by mere adsorption, of some injurious substance occurring in the culture medium. Such a substance might have been in the soil extract originally, or might be produced by the plant roots, or both suppositions might be true together. The third alternative proved to be the correct one.

TABLE I

DATA FOR EXPERIMENTS I TO IV.

Medium	Experiment I		Experiment II		Experiment III		Experiment IV	
	24 plants grown 19 days		24 plants grown 19 days		12 plants grown 17 days		36 plants grown 13 days	
	Total transpiration	Per cent. increase	Total transpiration	Per cent. increase	Total transpiration (av. of 2 cultur's)	Per cent. increase	Total transpiration	Per cent. increase
Untreated soil extract	154	....	(1) 33	....	51	....	(1) 68	....
Do.+tri-calc. phosphate	182	18.1	....	....	....	....	....	....
Do.+calcium carbonate	191	24.0	(2) 81	145.4	....	....	....	....
Do.+ferric hydrate	184	19.5	(3) 110	233.3	187	266.6	(2) 176	158.8
Do.+aluminum hydrate	187	21.4	....	....	....	....	....	....
Do.+carbon black	....	....	(4) 69	109.0	154	201.9	(3) 139	104.4
Do.+magnes. carbonate	....	....	(5) 73	121.2	....	....	....	....
Do.+barium carbonate	....	....	(6) 78	136.3	....	....	....	....
Do.+quartz flour	....	....	....	....	87	70.5	....	....
Do.+ferric hydrate, carbon black, and calcium carbonate	....	....	....	....	....	....	(4) 221	225.0

To obtain evidence in this regard, Experiment V was carried out. Four different soil extracts were shaken with carbon black, filtered, and then used as culture media, comparison being made with controls in untreated extracts. The four soils were of two types, a good and a poor soil of the Cecil clay type, and a good and a poor of the Leonardtown loam type, the former from Statesville, N. C., and the latter from Leonardtown, Md. Chemical analysis both of the aqueous extracts and of the solution obtained by digestion with hydrochloric acid, fails to show any material difference between the good and the poor varieties of these soils, although they are agriculturally quite different. These cultures were grown under the direction of Mr. F. D. GARDNER, in charge of the Division of Soil Management, of this Bureau. They consisted of forty-eight plants and were grown for fifteen days. The results are given in Table II; percentage increases are given for each treated extract compared with the same extract untreated considered as unity.

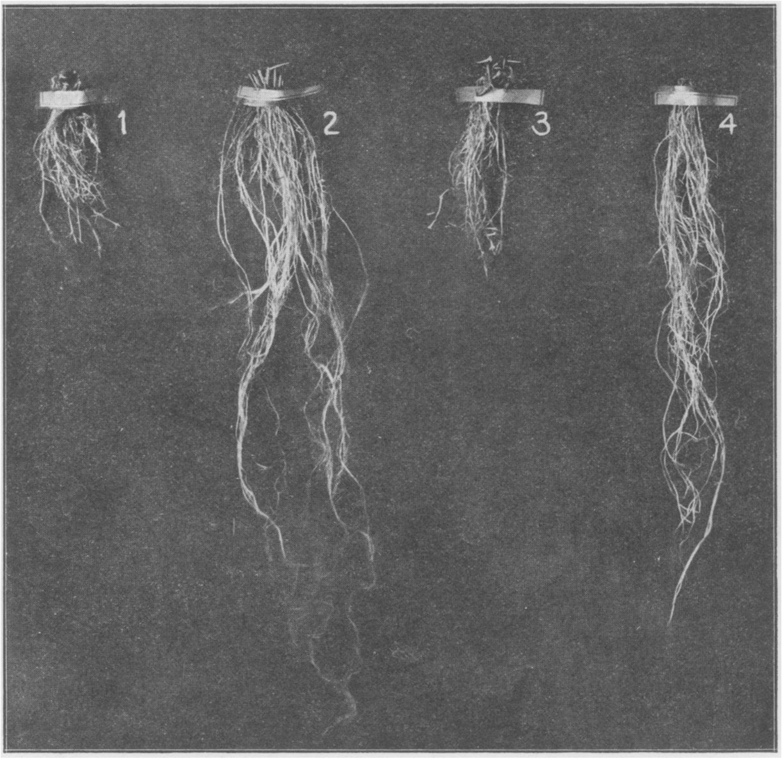

FIG. 3.—Experiment V; 48 wheat plants grown 15 days. 1, extract of Cecil clay, good; 2, the same, filtered from carbon black; 3, extract of Cecil clay, poor; 4, the same, filtered from carbon black.

It will be seen from this table that the extract of good Cecil clay

TABLE II.

DATA FOR EXPERIMENT V.

Culture no.	Medium	Total transpiration in grams	Per cent. increase
1	Extract of Cecil clay, good	485	.....
2	Do., carbon filtered	471	−2.9
3	Extract of Cecil clay, poor	407	.....
4	Do., carbon filtered	477	+17.2
5	Extract of Leonardtown loam, good	359	.....
6	Do., carbon filtered	407	+13.4
7	Extract of Leonardtown loam, poor	271	.....
8	Do., carbon filtered	349	+28.7

was not improved by treatment with carbon where the latter is filtered out. All the others were improved, although the improvement in the case of good Leonardtown loam was not as marked as that in case of the poor. The leaf development and general appearance of the plants were essentially in proportion to their transpiration. The roots showed the same acceleration with carbon treatment and filtering that had been observed in experiments in which the solid was left in the solution. *Fig. 3* shows the roots of cultures nos. 1, 2, 3 and 4. From this experiment and from similar ones it appears that a part at least of the injurious matter which is removed by carbon black is in the soil extract at the start.

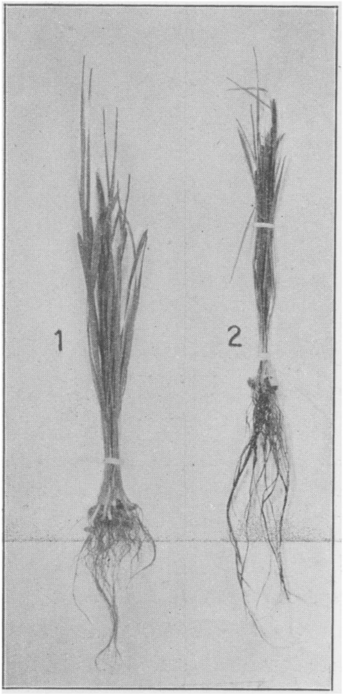

FIG. 4.—Twelve wheat plants grown 15 days. 1, redistilled water; 2, the same, with ferric hydrate.

A number of experiments were carried out, using water twice redistilled, first from potassium dichromate and sulfuric acid, then from alkaline potassium permanganate, both in glass boilers, condensation being carried on in a platinum tube. Upon this water the effects of carbon black and ferric hydrate were tested, the solids remaining in the water during the growth of the plants. The former gives little or no increase in transpiration, the latter a moderate increase, but both solids produce a marked acceleration in root growth. Twelve seedlings grown fifteen days in redistilled water with and without ferric hydrate are shown in *fig. 4*. No. 1 shows those from the untreated water, no. 2 those with the solid. The water here used probably did not contain injurious substances, and therefore the effect of the solids is most probably due to the removal of some injurious exudation arising from the plants. Further, distilled water in which plants have been grown for a number of days is found to give less growth upon replanting than does unused water, and the injurious effect of the used water is corrected by shaking with carbon black or ferric hydrate and filtering off the solid. Thus it seems that wheat seedlings do give off bodies from their roots which are toxic to themselves.

When the work so far recorded was practically completed, the appearance of TRUE and OGLEVEE'S paper[6] on somewhat similar experiments made it seem advisable to withhold publication until some further tests suggested by that paper could be made. These authors find that by the introduction of sand, filter paper, paraffin, or potato starch into solutions of copper sulfate in which seedlings of *Lupinus albus* are growing, the toxicity of the solute is remarkably decreased. By this means the killing concentration of the salt may be effectively reduced, according to the amount of the insoluble body present, either to a stimulating concentration or to one in which the physiological effect is not apparent. They reasoned that the solid absorbed the salt from the solution and in this way produced an effect closely paralleling that of simple dilution.

It was determined to test the effect of solids in solutions of sulfuric acid. Maize was here used instead of wheat. First, the strength of this acid necessary to prevent the growth of maize seedlings was

[6] TRUE, R. H. and OGLEVEE, C. C., The effect of the presence of insoluble substances on the toxic action of poisons. Science N. S. **19**:421–424. 1904. Also BOT. GAZETTE **39**:1–21. 1905. In this connection see also DANDENO, J. B., The relation of mass action and physical affinity to toxicity, etc. Am. Jour. Sci. **17**:437–458. 1904.

determined as lying between *n*/2750 and *n*/3250.[7] Then a series of varying concentrations of this acid on either side of the toxic limit was carried out, placing clear sand, quartz flour, filter paper, and paraffin shavings in the solutions. In no case was the apparent death limit modified by the presence of these substances. The death limit was also determined for a solution of sulfuric acid saturated with calcium sulfate, and for the same solution with an excess of calcium sulfate, but the solid again had no apparent effect.

With copper sulfate solutions carbon black was found to decrease the toxic effect, just as the authors above cited found to be true for the solids with which they worked. In order to bring out the effect of the carbon black, copper sulfate in the proportions of one and five parts per million of copper was added to the nutrient solution above described; a portion of the solution thus prepared was shaken with carbon black and then filtered, and wheat seedlings were grown in the treated and untreated solution. This series constitutes Experiment VI. Twenty-four plants composed a culture and the experiment lasted twelve days. The results are given in Table III.

TABLE III.

DATA FOR EXPERIMENT VI.

Culture no.	Medium	Total transpiration in grams
1...........	Nutrient solution...........	216.3
2...........	Do.+1 p.p.m. Cu..........	67.3
3...........	Do.+5 p.p.m. Cu..........	41.5
4...........	As 2, but carbon-treated....	140.2
5...........	As 3, but carbon-treated....	138.7

The growth of the plants was proportional to their transpiration. It is evident that the carbon removed sufficient copper to render carbon-treated solutions much less toxic than the untreated ones. This is a direct corroboration of the work of TRUE and OGLEVEE, but with another solid, and in this experiment the filtering out of the solid removes any possibility of its having any effect directly upon the roots. The explanation of these authors seems to be correct, as far as copper sulfate is concerned. The failure in the present instance to get the same effect with sulfuric acid may be due to the fact that to be injurious this substance must be at a much higher concentration than is needed in case of the copper salt, while the relative amount of solute absorbed by solids is much greater in dilute solutions than in more concentrated ones. TRUE and OGLEVEE suggest this explanation for their failure to get marked improvement by the use of sand in solutions of phenol and resorcinol.

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[7] This determination had been made previously, but was repeated for the present work. See CAMERON, F. K., and BREAZEALE, J. F., Toxic action of acids and salts on seedlings. Jour. Phys. Chem. **8**:1–13. 1904.

On account of the presence of toxic substances in distilled water as ordinarily prepared, from copper boiler and tin condenser, most workers with toxicity problems have used water redistilled in glass. In these laboratories the distilled water is often quite toxic to wheat seedlings, but its injurious effect is prevented if it is first shaken with carbon black or ferric hydrate and the solid filtered off. It is found that water so treated produces as good a growth of seedlings as does the most carefully prepared redistilled water.

It appears from these experiments (1) that extracts of certain soils are toxic to wheat seedlings in water culture, and that this toxicity is removed wholly or in part by carbon black, calcium carbonate, ferric hydrate, and other solids; (2) that the toxic substances of ordinary distilled water may be removed by ferric hydrate or carbon black; (3) that the roots of wheat seedlings give off substances which are toxic to themselves and that these substances can be made inactive by the presence of the last named solids in the culture medium; (4) that the presence of ferric hydrate and carbon black in the solution seemingly accelerates to a marked degree the development of roots, causing them to surpass the tops in growth.

The work here reported was done chiefly in the laboratories of the U. S. Department of Agriculture, Bureau of Soils, Washington, D. C. It was finished at the Rhode Island Agricultural Experiment Station, Kingston, R. I. I am indebted to Dr. F. K. CAMERON, and to Dr. B. E. LIVINGSTON, of the Bureau of Soils, for much valuable suggestion and advice.

U. S. DEPARTMENT OF AGRICULTURE,
Bureau of Soils,
Washington, D. C.